

STIC-ILL

NO

From: STIC-Biotech/ChemLib  
Sent: Thursday, October 09, 2003 6:01 AM  
To: STIC-ILL  
Subject: FW: In re: 10/005, 510 Journal article

467303

-----Original Message-----

From: Ford, Vanessa  
Sent: Wednesday, October 08, 2003 5:49 PM  
To: STIC-Biotech/ChemLib  
Subject: In re: 10/005, 510 Journal article

Please supply:

SO VET.MED. (76, NO.8, 1185-86, 1981).\*

SO Vet.Med. (82, No. 6, 646-50, 1987) 2 Tab. 23 Ref. \*

SO VETERINARY PARASITOLOGY, (MAY 1993) Vol. 47, No. 3-4, pp. 225-233.

SO Z PARASITENKD, (1983) 69 (1), 27-34.\*

SO Revista Brasileira de Parasitologia Veterinaria, (1995) Vol. 4, No. 1, pp.\*  
15-19.

SO Vet.Rec. (111, No. 18, 414-15, 1982) 2 Fig. 2 Tab. 12 Ref.\*

SO J PROTOZOOL, (1987) 34 (4), 398-402.

SO AM J TROP MED HYG, (1987) 36 (3), 505-508\*

Applied and Environmental Microbiology, (1995) Vol. 61, No. 2, pp.\*

SO Journal of Parasitology, (Feb., 1998) Vol. 84, No. 1, pp. 8-15.

SO Journal of the Egyptian Society of Parasitology, (Aug., 1999) Vol. 29, No.  
2, pp. 551-560. \*

SO FEMS Microbiology Letters, (1994) Vol. 118, No. 1-2, pp. 45-49.

SO J.Protozool. (35, No. 4, 583-89, 1988) 7 Fig. 2 Tab. 20 Ref. \*

SO AM J TROP MED HYG, (1987) 36 (3), 505-508. \*

SO J PARASITOL, (1976) 62 (2), 199-202.

Vanessa L. Ford  
Biotechnology Patent Examiner  
Office: CM1 8A16  
Mailbox: CM1 8E12  
Phone: 703.308.4735  
Art Unit: 1645

1931787

10/9  
10/9  
10/9

I, Dar FK, Paris J (1973) Serological studies  
trisons of antigenic types of *Trypanosoma*  
tsetse flies. Ann Trop Med Parasitol 67:31-43  
the effect of goat's blood. Trans R Soc

Experimental transmission to the crocodile.

i. 3. Life-cycle in the tsetse fly and in the

*Glossina* and its bearing upon the life-cycle  
Hyg 25:57-64

ogenesis of two soluble amino transferases.  
on

activities of eleven enzymes of bloodstream  
ared. Molec Biochem Parasitol 2:51-62  
istic isoenzymes of two amino transferases

ivarian trypanosomes from man and other  
28:521-534

fections of tsetse flies in Northern Nigeria  
J Res 14:265-288

924) Second report of the tsetse fly investiga-  
Entomol Res 15:1-28

*omua grayi* in the hindgut of *Glossina*. Proto-

acterisation of a cell line (BTC-32) from  
(Hemiptera: Reduviidae). Ann Trop Med

ishment of a cell line (XTC-2) from the  
-467

se in polymorphonuclear leukocytes. J Clin

cultivation in tsetse fly and vertebrate cell

## Excystation of *Isoospora suis* Biester, 1934 of Swine

David S. Lindsay,<sup>1</sup> William L. Current,<sup>1</sup> and John V. Ernst<sup>2</sup>

<sup>1</sup> Department of Zoology-Entomology, Auburn University, Alabama 36849, USA

<sup>2</sup> Regional Parasite Research Laboratory, USDA SEA-AR Auburn, Alabama 36830, USA

**Abstract.** The in vitro excystation of sporozoites of *Isoospora suis* Biester 1934 is described. Sporocysts of *I. suis* lack a Stieda body. Upon incubation in 0.75% sodium taurocholate or in 0.25% trypsin + 0.75% sodium taurocholate excystation solutions, sporozoites were released by separation of the sporocyst wall into four plates. Occasionally, the sporocyst wall did not separate completely but opened partially and released the sporozoite. At the time of excystation, sporozoites were short and broad but became elongated after 5 to 10 min in the excystation fluids. Elongate sporozoites measuring  $11.7 \times 3.8 \mu\text{m}$ , had a pointed anterior end and a nucleus located in the posterior half of the cell. Living sporozoites exhibited gliding movements, side-to-side flexion, and probed with their anterior ends. Incubation in 5.25% sodium hypochlorite removed the oocyst walls from most oocysts. Sporozoites did not excyst from sporocysts that were released during treatment with sodium hypochlorite.

### Introduction

Some species of *Isoospora* are similar to *Eimeria* spp. in that excystation involves dissolution of a Stieda body and escape of sporozoites through the small opening left in the end of the sporocyst wall (Duszynski and Brunson 1972; Speer and Duszynski 1975). In contrast, several species of *Isoospora* without a Stieda body in one end of their sporocysts have been shown to excyst by collapse of sporocyst wall plates (Speer et al. 1973; Duszynski and File 1974; Duszynski and Speer 1976).

Endogenous development (Lindsay et al. 1980) and sporogony (Lindsay et al. in press) of *Isoospora suis* Biester 1934 has been described. The present paper provides additional information about the life cycle of this economically important pathogen of neonatal pigs (Stuart et al. 1980; Eustis and Nelson 1981).

Offprint requests to: D.S. Lindsay

0044-3255/83/0069/0027/\$01.60

## Materials and Methods

Oocysts of *I. suis* were obtained from experimentally infected pigs, sporulated in 2.5% potassium dichromate solution (Lindsay et al. 1980) and stored at 4° C for 60 to 70 days before being used in the present studies. Oocysts were cleaned and concentrated using Sheather's sugar solution (Lindsay et al. 1980), washed by centrifugation, and suspended in Ringer's "warm-blooded" saline (RWS).

Groups of oocysts were processed by four different methods: (a) treatment with 5.25% sodium hypochlorite (NaOCl) for 15 min in an ice bath; (b) treatment with 2.63% NaOCl for 15 min in an ice bath; (c) grinding with a teflon coated tissue grinder to release the sporocysts; and (d) resuspending untreated oocysts in RWS and leaving them in this solution. Oocysts in groups 1 and 2 were washed four times and resuspended in RWS before addition of the test excystation solutions.

Untreated oocysts, oocysts treated with NaOCl, and sporocysts released by grinding were each incubated at 37° C in four different test solutions. The solutions were: (a) 75% sodium taurocholate (bile salt) in RWS, (b) 0.25% trypsin + 0.75% sodium taurocholate (trypsin-bile salt) in RWS, (c) 0.25% trypsin in RWS; and (d) RWS alone. Samples from each oocysts or sporocyst preparation in each test solution were examined by Nomarski interference contrast microscopy at 5-min intervals until excystation was complete. If sporozoites were not released within 2 h, the solution was considered incapable of stimulating excystation. To judge the efficacy of excystation of untreated oocysts and of oocysts treated with NaOCl, 200 of these stages were examined after 2 h incubation at 37° C, and the percentage of oocysts which had released sporozoites was determined.

Fifty living sporozoites were measured at the end of 2-h incubation in the trypsin-bile salt solution. Measurements were also made of methanol-fixed and Giemsa-stained sporozoites. All measurements were made with a calibrated ocular micrometer. In all instances the means are followed by ranges in parentheses.

To test the effects of age of oocyst on excystation, oocysts stored in 2.5% potassium dichromate solution for 30 and 260 days were also processed like group c above, and incubated in trypsin-bile salt solution.

## Results

Some untreated oocysts had walls which appeared wrinkled or cracked when examined before addition of excystation solutions (Fig. 1). Sporocysts inside these oocysts were morphologically normal. Excystation solutions did not cause any visible change in the oocyst walls.

Treatment with 5.25% or 2.63% NaOCl for 15 min removed the oocyst walls from most oocysts. However, some remained completely intact and some had only intact inner oocyst walls surrounding the sporocysts (Fig. 2). Most sporocysts released from oocysts treated with 5.25% or 2.63% NaOCl appeared morphologically normal but a few had areas in the sporocyst wall that were collapsed. All sporocysts released from NaOCl-treated oocysts failed to excyst when incubated in any of the excystation solutions. Also sporozoites freed mechanically from thus treated sporocysts were non-motile and appeared dead. However, sporocysts not released from oocysts treated with 5.25% or 2.63% NaOCl behaved as those in untreated oocysts when incubated in excystation solutions.

Excystation occurred in untreated, as well as in 5.25% and 2.63% NaOCl-treated oocysts, with one or both walls intact, after incubation for

10 to 20 min in bile salt in the former solution, with 5.25% NaOCl, and sporozoites from their sporocysts in the latter solution, 26% of untreated oocysts, 5.25% NaOCl, and 32% of sporocysts from their sporocysts freed sporocysts after 5 min in the former solution. After 2-h incubation, 95% of the mechanically freed sporocysts, 100% of oocysts treated with 2.63% NaOCl, or more than 100% of oocysts in the 0.25% trypsin solution.

No differences were observed between sporocysts which were 30- or 60- to 70- days old and those released sporozoites after 2 h incubation.

Before excystation, sporozoites showed a tumbling movement. After excystation, the cyst wall could be seen separating from the sporocyst walls then separating from the sporozoites (Figs. 4, 5). Less than 10% of sporozoites emerged from oocysts after excystation of sporozoites was observed. Occasionally a sporozoite excysted by itself, released sporozoites by themselves, released from sporocysts, served moving freely in a break in the oocyst wall, oocysts with intact walls.

Freshly excysted sporozoites were motile (Fig. 10). Five to ten micrometers long, with a flagellum and highly motile. The head was rounded posteriorly and a rounded posterior half. Small refractile granules in the cytoplasm, flexion, and a probing movement were seen in any of these stages (10-13) × 3.8 (3-5) µm.

Giemsa-stained sporozoites showed no regard to the position of the head in the cytoplasm. The head was not evident in Giemsa-stained sporozoites. Fifty Giemsa-stained sporozoites

ally infected pigs, sporulated in 2.5% potassium dichromate and stored at 4°C for 60 to 70 days before being cleaned and concentrated using Sheather's flotation, centrifugation, and suspended in Ringer's solution.

different methods: (a) treatment with 5.25% sodium hypochlorite; (b) treatment with 2.63% NaOCl in a teflon coated tissue grinder to release the sporozoites in RWS and leaving them in this solution. (c) mechanical disruption and resuspended in RWS before addition

of NaOCl, and sporocysts released by grinding were used in the solutions. The solutions were: (a) 75% sodium hypochlorite + 0.75% sodium taurocholate (trypsin-bile salt solution); (b) 75% sodium hypochlorite + 0.75% sodium taurocholate (trypsin-bile salt solution); (c) RWS alone. Samples from each oocyst were examined by Nomarski interference contrast microscopy. If sporozoites were not released after 2 h of stimulating excystation. To judge the effectiveness of oocysts treated with NaOCl, 200 of these were incubated at 37°C, and the percentage of oocysts which

released sporozoites at the end of 2-h incubation in the trypsin-bile salt solution, ethanol-fixed and Giemsa-stained sporozoites. In all instances the means

excystation, oocysts stored in 2.5% potassium dichromate and processed like group c above, and incubated

appeared wrinkled or cracked when in the solutions (Fig. 1). Sporocysts inside oocysts remained normal. Excystation solutions did not damage the walls.

NaOCl for 15 min removed the oocysts which remained completely intact and surrounding the sporocysts (Fig. 2). Sporocysts treated with 5.25% or 2.63% NaOCl released sporozoites from a few had areas in the sporocyst wall. Sporozoites released from NaOCl-treated oocysts were found in any of the excystation solutions. Sporozoites from thus treated sporocysts were non-motile. Sporocysts not released from oocysts behaved as those in untreated oocysts.

as well as in 5.25% and 2.63% NaOCl, both walls intact, after incubation for

10 to 20 min in bile salt or trypsin-bile salt solution. After 2-h incubation in the former solution, 35% of untreated oocysts, 11% of oocysts treated with 5.25% NaOCl, and 28% of those treated with 2.63% NaOCl released sporozoites from their sporocysts. After 2-h incubation in trypsin-bile salt solution, 26% of untreated oocysts, 15% of those treated with 5.25% NaOCl, and 32% of those treated with 2.63% NaOCl released sporozoites from their sporocysts. Sporozoites were released from mechanically freed sporocysts after 5–10 min incubation in bile salt or trypsin-bile salt solution. After 2-h incubation in either of these solutions, approximately 95% of the mechanically freed sporocysts released their sporozoites. No excystation occurred in untreated oocysts, intact oocysts treated with 5.25% of 2.63% NaOCl, or mechanically freed sporocysts after 2 h in RWS or in the 0.25% trypsin solution.

No differences were seen in the method of releasing sporozoites from sporocysts which were 30, 60 to 70, or 260 days-old. Approximately 95% of the 30- or 60-to 70-day-old sporocysts and 90% of those 260-days-old released sporozoites after incubation in trypsin-bile salt solution.

Before excystation, sporozoites swelled slightly and exhibited intermittent tumbling movements inside the sporocysts. Indentations in the sporocyst wall could be seen shortly after these movements began (Fig. 3). Most sporocyst walls then separated quickly into four plates releasing the sporozoites (Figs. 4, 5). Less frequently, the sporocyst wall opened only partially and sporozoites emerged through the opening (Figs. 6, 7). Simultaneous excystation of sporozoites from both sporocysts within an oocyst was not observed. Occasionally an oocyst was seen with one sporocyst from which sporozoites excysted by complete collapse of plates and the other which released sporozoites by partial separation of the plates (Fig. 8). Sporozoites released from sporocysts that were surrounded by an oocyst wall were observed moving freely inside the oocyst. Eventually, they escaped through a break in the oocyst wall. Sporozoites apparently could not escape from oocysts with intact walls (Fig. 9).

Freshly excysted sporozoites were short and broad; they were weakly motile (Fig. 10). Five to 10 min after excystation, sporozoites became elongate and highly motile. Elongate sporozoites (Fig. 11) had a pointed anterior end and a rounded posterior end, with a nucleus located within the posterior half. Small refractile granules were scattered throughout these stages. Movements of such sporozoites consisted of rapid forward gliding, side-to-side flexion, and a probing action with the anterior end. No refractile bodies were seen in any of these stages. Fifty elongated sporozoites measured  $11.7 (10-13) \times 3.8 (3-5) \mu\text{m}$ .

Giemsa-stained sporozoites (Fig. 12) resembled living sporozoites with regard to the position of the nucleus and the presence of scattered granules in the cytoplasm. The pointed anterior end seen in living sporozoites was not evident in Giemsa-stained organisms, in which no refractile bodies were noted. Fifty Giemsa-stained sporozoites measured  $9.9 (8-12) \times 2.8 (2-5) \mu\text{m}$ .

# Discussion

The abnormalities (wrinkled walls of untreated *I. suis*) were probably due to storage abnormalities were not observed (Lindsay et al. in press). In the oocyst wall had no process, since approximately oocysts stored for 30 or stored for 260 days released solution. In addition, oocysts produced clinical comparable from that produced

Figs. 1-12. Nomarski interference graphs of oocysts, sporocysts, (Figs. 3-10), and after (Figs. 11-12).

Fig. 1. Untreated oocyst showing cyst wall.

Fig. 2. Oocyst pretreated with of the removed outer wall *OW*.

Fig. 3. Mechanically freed sporocyst.

Fig. 4. Untreated oocyst in which to sites where plates *PL* are situated the oocyst.

Fig. 5. Sporocyst wall plates removed sporocyst.

Fig. 6. Oocyst, pretreated with one which has partially opened *OP*.

Fig. 7. Mechanically freed sporocyst.

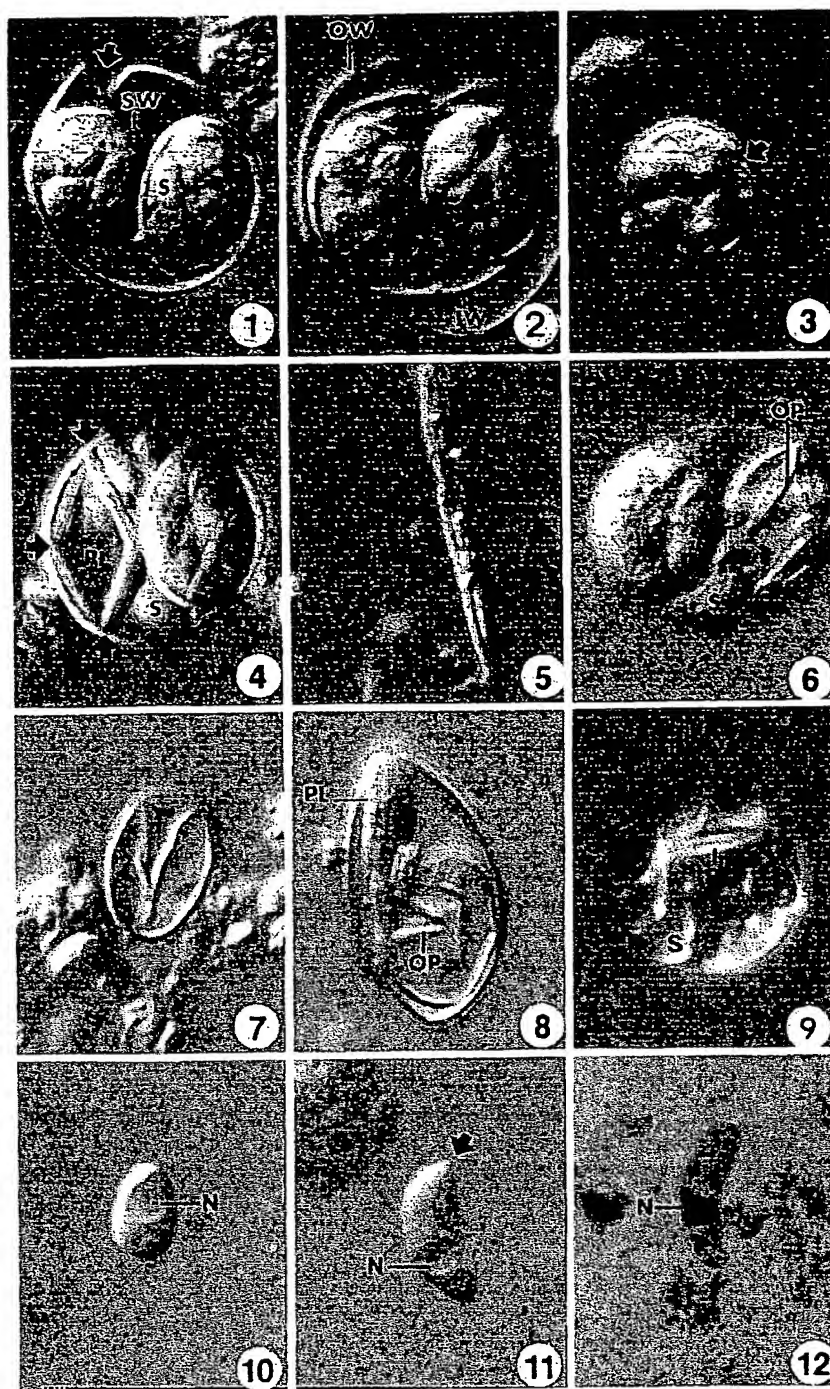
Fig. 8. Untreated oocyst containing with partly opened wall plates *OP*.

Fig. 9. Untreated oocyst 2 h after but the released sporozoites *S* have.

Fig. 10. Freshly excysted, short,

Fig. 11. Elongate sporozoite with posterior half of the cell.

Fig. 12. Giemsa-stained sporozoite nucleus *N* located just posterior to anterior end.



### Discussion

The abnormalities (wrinkles and/or cracks) observed in some of the oocyst walls of untreated *I. suis* oocysts before addition of excystation solutions were probably due to storage and concentration techniques. These abnormalities were not observed in oocyst walls of freshly sporulated oocysts (Lindsay et al. in press). In the present study, the morphologic abnormalities in the oocyst wall had no apparent physiologic effect on the excystation process, since approximately 95% of the sporocysts freed mechanically from oocysts stored for 30 or 60–70 days and approximately 90% from those stored for 260 days released sporozoites when incubated in trypsin-bile salt solution. In addition, oocysts stored for 260 days in 2.5% potassium dichromate produced clinical coccidiosis in susceptible piglets which was indistinguishable from that produced by inoculating the same number of oocysts

Figs. 1–12. Nomarski interference contrast (Figs. 1–11) and bright field (Fig. 12) photomicrographs of oocysts, sporocysts, and sporozoites of *Isospora suis* before (Figs. 1, 2), during (Figs. 3–10), and after (Figs. 11, 12) incubation in excystation solution. All figures,  $\times 1,600$

Fig. 1. Untreated oocyst showing a break (arrow) in the oocyst wall. *S* sporozoite; *SW* sporocyst wall.

Fig. 2. Oocyst pretreated with 5.25% NaOCl. Note the intact inner wall *IW* and portion of the removed outer wall *OW*.

Fig. 3. Mechanically freed sporocyst showing an indentation (arrow) in the sporocyst wall.

Fig. 4. Untreated oocyst in which one sporocyst has separated into four plates. Arrows point to sites where plates *PL* are still joined. Several released sporozoites *S* can be seen within the oocyst.

Fig. 5. Sporocyst wall plates remaining after release of sporozoites from a mechanically freed sporocyst.

Fig. 6. Oocyst, pretreated with 5.25% NaOCl, containing an intact sporocyst and another one which has partially opened and released its sporozoites *S*. Opening in sporocyst wall *OP*.

Fig. 7. Mechanically freed sporocyst which has opened partially and released its sporozoites.

Fig. 8. Untreated oocyst containing one sporocyst with collapsed wall plates *PL* and another with partly opened wall plates *OP*.

Fig. 9. Untreated oocyst 2 h after incubation. Both sporocysts have collapsed wall plates *PL*, but the released sporozoites *S* have not left the oocyst.

Fig. 10. Freshly excysted, short, broad sporozoite with a prominent nucleus *N*.

Fig. 11. Elongate sporozoite with a pointed anterior end (arrow) and a nucleus *N* in the posterior half of the cell.

Fig. 12. Giemsa-stained sporozoite showing granules scattered within the cytoplasm and a nucleus *N* located just posterior to the middle of the cell. Note the absence of a pointed anterior end.



stored for 30 or 60–70 days. Similarly, storage in potassium dichromate of *I. canis* oocysts for 56 days (Speer et al. 1973) and *I. arctopitheci* oocysts for 100 days (Duszynski and Speer 1976) had no adverse effects on sporocyst structure or on the excystation process.

Complete removal of the oocyst walls by 5.25% or 2.63% NaOCl-treatment indicates that oocysts of *I. suis* are more sensitive to NaOCl than these forms of many species of *Eimeria* and *Isospora* in which similar treatment removes only the outer wall (Nyberg and Knapp 1970; Roberts et al. 1970; Speer et al. 1973; Dubey 1975, 1979; Dubey and Fayer 1976). Pretreatment of oocysts of *I. felis* (Fayer and Thompson 1974), *I. ohioensis* (Dubey 1975), *I. rivolta* (Dubey 1979), and *I. bigemina* (Dubey and Fayer 1976) with NaOCl did not adversely affect their infectivity for, and development in susceptible hosts and cell cultures. In the present study, direct exposure of *I. suis* sporocysts to 5.25% or 2.63% NaOCl solutions apparently killed the sporozoites, as they were nonmotile and failed to excyst when incubated in the excystation solutions. Also, when sporocysts previously exposed to 5.25% or 2.63% NaOCl were washed, suspended in RWS, and mechanically ruptured, the sporozoites released were nonmotile and appeared dead. Some protection from the adverse effects of NaOCl was provided for sporocysts within oocysts which retained the inner wall, since approximately 11% contained sporocysts capable of releasing viable sporozoites when placed in excysting solutions. Storage of sporocysts of *Sarcocystis cruzi* in a 1% NaOCl solution greatly reduced the percent of excystation in comparison to that recorded for sporocysts stored in distilled water or in a balanced salt solution (Leek and Fayer 1979). Results obtained with *I. suis* (present study) and those derived from studies of *S. cruzi* (Leek and Fayer, 1979) indicate that sporocysts of both these species are more sensitive to NaOCl than are the oocysts of most species of *Eimeria*.

The excystation process of *I. suis* is similar to that described previously for *I. canis* (Speer et al. 1973), *I. endocallimici* (Duszynski and File 1974), *I. arctopitheci* and *I. bigemina* (Duszynski and Speer 1976), as well as for *Toxoplasma gondii* (Christie et al. 1978) and *Sarcocystis* spp. (Box et al. 1980), all of which lack a Stieda body and excyst by separation of sporocyst wall plates. Simultaneous release of sporozoites from both sporocysts within an oocyst was seen in *I. canis* (Speer et al. 1973) and *T. gondii* (Christie et al. 1978), but not in *I. endocallimici* (Duszynski and File 1974) or in *I. suis* (present study). Intact oocysts of *I. endocallimici* (Duszynski and File 1974) and *I. arctopitheci* (Duszynski and Speer 1976) did not excyst when exposed to trypsin-bile salt solutions. Untreated oocysts of *I. canis* excysted after prolonged incubation in excystation solution, whereas NaOCl-treated oocysts excysted after a much briefer incubation (Speer et al. 1973). In the present study, the time of initiation of excystation was the same for untreated and 5.25% and 2.63% NaOCl-treated oocysts. The wrinkles and cracks observed in the walls of some untreated oocyst (Cf. Fig. 1) probably allowed entrance of excysting solution which then stimulated excystation. No excystation occurred within oocysts with normal-appearing walls and no visible alteration in the oocyst wall occurred when the oocysts were incubated

in excystation solutions. to be the only stimulus ne tions were reported for et al. 1978). Trypsin solu of *I. suis* (present study, 1973). Roberts et al. (19 was the only treatment ne oocysts of *Eimeria callosa* solution alone caused m not excystation. In sporc accomplished by separation a proteolytic enzyme is n that movement of the sp sporocyst wall plates, ca zoites.

Sporozoites of *I. suis* (Speer 1976) in that both cysts, but become elonga of *I. arctopitheci* sporozo cytoplasm which disapp the short or elongate form

Of the coccidia of wa whose sporocysts contain whose sporocysts are co et al. 1980). Since excyste Box et al. (1980) propos in the Eimeridae and th in the Sarcocystidae. If th to the genus *Cystoisospora* *Cystoisospora* was based hosts. Since *I. suis* has n of this parasite would be

**Acknowledgements.** This investment No. 58-7B30 1-265, Al by Research grant No. SEA-59

## References

- Box ED, Marchiondo AA. D sporocysts from passerine *Isospora*. J Parasitol 66:68
- Christie E, Pappas PW, Dubey JP (1975) *Isospora* oocysts. J Protozool 25:438
- Dubey JP (1975) *Isospora* oocysts. J Parasitol 61:462-465
- Dubey JP (1979) Life cycle of *Isospora*. J Parasitol 65:433-443
- Dubey JP, Fayer R (1976) Development of *Isospora*. Parasitology 73:371-380

, storage in potassium dichromate (al. 1973) and *I. arctopitheci* oocysts) had no adverse effects on sporocyst

ls by 5.25% or 2.63% NaOCl-treat- are more sensitive to NaOCl than and *Isospora* in which similar treat- erg and Knapp 1970; Roberts et al. 1979; Dubey and Fayer 1976). Pre- and Thompson 1974), *I. ohioensis* and *I. bigemina* (Dubey and Fayer ect their infectivity for, and develop- ltures. In the present study, direct or 2.63% NaOCl solutions apparent- nonmotile and failed to excyst when . Also, when sporocysts previously re washed, suspended in RWS, and s released were nonmotile and ap- s adverse effects of NaOCl was pro- which retained the inner wall, since sts capable of releasing viable sporo- is. Storage of sporocysts of *Sarcocys*- ly reduced the percent of excystation orocysts stored in distilled water or Fayer 1979). Results obtained with d from studies of *S. cruzi* (Leek and both these species are more sensitive species of *Eimeria*.

similar to that described previously *callimici* (Duszynski and File 1974), ski and Speer 1976), as well as for 18) and *Sarcocystis* spp. (Box et al. and excyst by separation of sporocyst rozoites from both sporocysts within et al. 1973) and *T. gondii* (Christie ci (Duszynski and File 1974) or in f *I. endocallimici* (Duszynski and File nd Speer 1976) did not excyst when Jntreated oocysts of *I. canis* excysted ion solution, whereas NaOCl-treated incubation (Speer et al. 1973). In the xcystation was the same for untreated d oocysts. The wrinkles and cracks l oocyst (Cf. Fig. 1) probably allowed en stimulated excystation. No excys- ormal-appearing walls and no visible d when the oocysts were incubated

in excystation solutions. In the present study, bile salt solution was shown to be the only stimulus necessary for excystation of *I. suis*. Similar observa- tions were reported for *I. canis* (Speer et al. 1973) and *T. gondii* (Christie et al. 1978). Trypsin solution alone was not sufficient to cause excystation of *I. suis* (present study) or of *I. canis* in a previous study (Speer et al. 1973). Roberts et al. (1970) reported that incubation in trypsin solution was the only treatment necessary for excystation of mechanically freed spor- ocysts of *Eimeria callospermophili* and *E. larimerensis* and that a bile salt solution alone caused movement of the sporozoites within sporocysts but not excystation. In sporocysts which lack a Stieda body, excystation is ac- complished by separation of sporocyst wall plates. Since treatment with a proteolytic enzyme is not necessary for excystation of *I. suis*, it is possible that movement of the sporozoites inside sporocyst exerts pressure on the sporocyst wall plates, causing them to separate and to release the sporo- zoites.

Sporozoites of *I. suis* resemble those of *I. arctopitheci* (Duszynski and Speer 1976) in that both are short and broad upon release from the sporo- cysts, but become elongate after a brief incubation period. The short forms of *I. arctopitheci* sporozoites contain a relatively large dense body in the cytoplasm which disappears after elongation. No such body was seen in the short or elongate forms of *I. suis* sporozoites (present study).

Of the coccidia of warm blooded animals with known life cycles, those whose sporocysts contain a Stieda body are homoxenous whereas those whose sporocysts are composed of plates have an intermediate host (Box et al. 1980). Since excystation structures appear to correlate with life cycles, Box et al. (1980) proposed that *Isospora* spp. with Stieda bodies be placed in the Eimeridae and that *Isospora* spp. without Stieda bodies be placed in the Sarcocystidae. If this proposal is accepted, *I. suis* would be transferred to the genus *Cystoisospora*. Frenkel's (1977) original description of the genus *Cystoisospora* was based on the presence of monozyotic cysts in intermediate hosts. Since *I. suis* has not been shown to have such cysts, reclassification of this parasite would be premature.

**Acknowledgements.** This investigation was supported in part by General Cooperative Agree- ment No. 58-7B30 1-265, Alabama Agricultural Experiment Station and USDA, AR and by Research grant No. SEA-59201120510 from U.S.D.A. (to WLC).

## References

- Box ED, Marchiondo AA, Duszynski DW, Davis CP (1980) Ultrastructure of *Sarcocystis* sporocysts from passerine birds and opossums: comments on classification of the genus *Isospora*. *J Parasitol* 66:68-74
- Christie E, Pappas PW, Dubey JP (1978) Ultrastructure of excystment of *Toxoplasma gondii* oocysts. *J Protozool* 25:438-443
- Dubey JP (1975) *Isospora ohioensis* sp. n. proposed for *I. rivolta* of the dog. *J Parasitol* 61:462-465
- Dubey JP (1979) Life cycle of *Isospora rivolta* (Grassi 1879) in cats and mice. *J Protozool* 26:433-443
- Dubey JP, Fayer R (1976) Development of *Isospora bigemina* in dogs and other mammals. *Parasitology* 73:371-380



- Duszynski DW, Brunson JT (1972) The structure of the oocyst and the excystation process of *Isoospora marquardtii* sp.n. from the Colorado pika, *Ochotona princeps*. J Protozool 19:257-259
- Duszynski DW, File SK (1974) Structure of the oocyst and excystation of sporozoites of *Isoospora endocallimici* n.sp., from the marmoset *Callimico goeldii*. Trans Amer Micros Soc 93:403-408
- Duszynski DW, Speer CA (1976) Excystation of *Isoospora arcotopithecii* Rodhain, 1933 with notes on a similar process in *Isoospora bigemina* (Stiles 1891) Luhe 1906. Z Parasitenk 48:191-197
- Eustis SL, Nelson DT (1981) Lesions associated with coccidiosis in nursing piglets. Vet Pathol 18:21-28
- Fayer R, Thompson DE (1974) *Isoospora felis*: development in cultured cells with some cytological observations. J Parasitol 60:160-168
- Frenkel JK (1977) *Besnoitia wallacei* of cats and rodents: with a reclassification of other cyst-forming isosporoid coccidia. J Parasitol 63:611-628
- Leek RG, Fayer R (1979) Survival of sporocysts of *Sarcocystis* in various media. Proc Helminthol Soc Wash 46:151-154
- Lindsay DS, Current WL, Ernst JV (1982) Sporogony of *Isoospora suis* Biester 1934 of swine. J Parasitol, in press
- Lindsay DS, Stuart BP, Wheat BE, Ernst JV (1980) Endogenous development of the swine coccidium, *Isoospora suis* Biester 1934. J Parasitol 66:771-779 (1980)
- Nyberg PA, Knapp SE (1970) Effect of sodium hypochlorite on the oocyst wall of *Eimeria tenella* as shown by electron microscopy. Proc Helminthol Soc Wash 37:32-36
- Roberts WL, Speer CA, Hammond DM (1970) Electron and light microscope studies of the oocysts walls, sporocysts, and excysting sporozoites of *Eimeria callospermophilii* and *E. lurimereensis*. J Parasitol 56:918-926
- Speer CA, Duszynski DW (1975) Fine structure of the oocyst walls of *Isoospora serini* and *Isoospora canaria* and excystation of *Isoospora serini* from the canary, *Serinus canarius* L. J Protozool 22:476-481
- Speer CA, Hammond DM, Mahri JL, Roberts WL (1973) Structure of the oocyst and oocyst walls and excystation of sporozoites of *Isoospora canis*. J Parasitol 59:35-40
- Stuart BP, Lindsay DS, Ernst JV, Gosser HS (1980) *Isoospora suis* enteritis in piglets. Vet Pathol 17:84-93

Accepted June 20, 1982

## The Chetotaxy of of *Opisthioglyphe* (Trematoda, Plagi)

Dieter Bock

Universität Ulm, Abt. Ökologie  
Oberer Eselsberg, D-7900 Ulm

**Abstract.** The distribution of the cercariae was studied for the cercariae that emerge from the fresh water according to the method of papillae were demonstrated. The results were identified as *Opisthioglyphe corneus* from a perfect; laboratory-reared distributed symmetrically of the trematode with by the chetotaxy of the cercaria from the system of the cercariae characteristics with other cercariae like the chetotaxy, em-

### Introduction

The sensory receptors (tegumental papillae) of the cercariae lie on the body surface. Their distribution is characteristic of a trematode species. It is regarded as unchanged and has a high taxonomical value. A taxonomical investigation of the cercariae from the beginning, and information about the cercariae of *Opisthioglyphe locellus* *corneus* L. as first host and insects as second intermediate